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### Citation for published version:

Kaba, J, Nowicki, M, Frymus, T, Nowicka, D, Witkowski, L, Szalus-Jordanow, O, Czopowicz, M & Thrusfield, M 2011, 'Evaluation of the risk factors influencing the spread of caseous lymphadenitis in goat herds', *Polish journal of veterinary sciences*, vol. 14, no. 2, pp. 231-237. <https://doi.org/10.2478/v10181-011-0035-6>

### Digital Object Identifier (DOI):

[10.2478/v10181-011-0035-6](https://doi.org/10.2478/v10181-011-0035-6)

### Link:

[Link to publication record in Edinburgh Research Explorer](#)

### Document Version:

Publisher's PDF, also known as Version of record

### Published In:

Polish journal of veterinary sciences

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DOI 10.2478/v10181-011-0035-6

Original article

# Evaluation of the risk factors influencing the spread of caseous lymphadenitis in goat herds

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## Abstract

Epidemiological studies on caseous lymphadenitis were carried out in Poland in 1996 and 2002 among goat herds covered by a milk recording program. Between-herd seroprevalence was 13.2% in 1996 and increased to 62.5% in 2002. The average size of seropositive herds was statistically significantly higher than that of seronegative ones, however there was no statistically significant difference in the age between the herds. A statistically significant prevalence ratio (PR) was identified and relevant attributable risk for exposed animals ( $AR_{exp}$ ) was calculated for the following risk factors: presence of seropositive males in a herd ( $PR=8.350$ ;  $AR_{exp}=0.651$ ), presence of superficial abscesses in animals ( $PR=6.142$ ;  $AR_{exp}=0.620$ ), presence of respiratory signs ( $PR=2.900$ ;  $AR_{exp}=0.393$ ), presence of animals in poor condition in a herd ( $PR=2.774$ ;  $AR_{exp}=0.390$ ) and occurrence of reproductive failures in a herd ( $PR=1.798$ ;  $AR_{exp}=0.230$ ). Purchase of animals from abroad, mastitis and husbandry conditions (housing system, grazing system, hygienic conditions) were not shown to be statistically significant risk factors.

**Key words:** caseous lymphadenitis, *Corynebacterium pseudotuberculosis*, abscesses, risk factors, goat

## Introduction

Caseous lymphadenitis (CLA) is a worldwide infectious, chronic, emaciating disease of small ruminants, caused by *Corynebacterium pseudotuberculosis*.

The disease has been recorded in Poland (Kempski and Kneblewski 1982). The information on the prevalence of CLA is limited. Recently, results of reliable large-scale epidemiological study on the goat population in Brazil have been published (Seyffert et al.

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2010). Epidemiological studies carried out in other countries have usually been limited to the disease detection (Connor et al. 2000, Chirino-Zarraga et al. 2006). Some disease surveys were based on such small samples that results could not be considered reliable (Hein and Cargill 1981, Batey 1986).

Caseous lymphadenitis is responsible for substantial economic losses in goat herds and sheep flocks (Williamson 2001). The disease is characterized by a sub-acute or chronic course, leading to the development of abscesses in the lymph nodes or internal organs. In goats, abscesses are found mostly in superficial lymph nodes. The disease is usually introduced into a herd by purchase of new animals (Kuria et al. 2001) and is spread within a herd mainly by direct contact between animals. Either damaged or undamaged skin and mucous membranes are the main portals of infection, followed by the respiratory tract and, less frequently, alimentary tract (Williamson 2001, Dorella et al. 2006).

Caseous lymphadenitis is a notifiable disease in Poland. However, as there are no mandatory eradication programs, attempts to control the disease are rarely undertaken. Such a situation may facilitate unlimited spread of CLA both between and within herds. Identification of statistically significant risk factors for the disease allows estimation of the attributable risk (Thrusfield 2007). This parameter shows the impact exerted by a risk factor on disease occurrence in a herd. Such information is a key to design a disease control program. It also provides practical assistance in reducing the risk of contracting the disease when new animals are introduced into a herd.

The objective of this study was to evaluate risk factors for the spread of CLA in a population of goats covered by the Polish Sheep Breeders Association's milk recording program.

## Materials and Methods

### Sampling method

Serum was collected during two disease surveys including goats covered by the milk recording program in 1996 and 2002. Serum was collected from animals older than 12 months. There were 5035 such goats in 348 herds in 1996 so a one-stage cluster sampling protocol was applied to estimate within-herd as well as between-herd prevalence (Thrusfield 2007). The number of herds required to estimate a pre-specified prevalence with pre-specified precision was calculated for 95% level of confidence. Expected between-herd prevalence was predetermined to be 15%, based on information provided by farmers. Desired absolute precision of 5% was assumed. Moreover an adjustment was applied because both the number of

herds and the number of animals in the population were small (Thrusfield 2007). The final number recovered from the calculation was 65 but it was increased to 80 to cope with any organizational or technical handicaps that might result in a shortfall of samples, and these were randomly selected from 348 herds in the sampling frame. The survey conducted in 1996 finally covered 76 of 80 initially selected herds (21.78% of herds covered by the milk recording program) in all 16 provinces of Poland. Size of herds varied from one to 240 goats. Blood samples were collected from all animals in a herd. In this manner 1004 females (20.5% of females covered by milk recording) and 70 males (55.1%) were tested. There were 942 females and 63 males at the age of 1-5 years, while only 62 females and 7 males older than five years. In 2002 samples were collected only to estimate between-herd prevalence. As only 68 herds were covered by the milk recording program at the time, all were included in the study. The number of goats blood-sampled in each herd was enough to ensure that at least one seropositive animal was detected in each herd with 95% probability, at a minimum within-herd prevalence 10%. The formula commonly accepted in veterinary epidemiology was applied (Thrusfield 2007). Required sample size ranged from 1 for the smallest herd counting only one animal, to 28 in a herd of 347 goats. The survey carried out in 2002 finally included 65 of 68 herds covered by the milk recording program (95.6%) in all 16 provinces. One thousand three hundred eighty six females (36.6% of females covered by the milk recording program) and 91 males (72.8%) were tested. Prevalence calculated for females in population (Table 2) was only an approximation, because in 2002 the sample was too small to evaluate within-herd prevalence for females properly.

In each herd, samples were collected from all male goats (if present on a farm at the time of sampling), whereas the required number of females, for either prevalence estimation (1996) or disease detection (2002) were randomly selected from the sampling frame.

### Serological tests

Approximately 10 ml of blood were collected from the external jugular vein into sterile containers and centrifuged at 3000 rpm for 15 minutes within 24-48 hours. Then serum was divided into 100 µl aliquots and stored at -20°C. Serological examination was carried out using an indirect enzyme-linked immunosorbent assay (ELISA), intended for detection of antibodies to the phospholipase D (PLD) exotoxin of *C. pseudotuberculosis*. Sensitivity of the test was 85% and specificity 96% (Kaba et al. 2001). A herd was recog-

nized as positive when at least one seropositive female was found.

### Questionnaire

A standardized face-to-face interview was carried out with the owner of each herd in order to collect following information: the date a herd was established; the number of animals in a herd; purchases of animals from abroad during the former five years (yes or no); hygiene conditions (classified as good or poor); rearing and housing system (free-stall or stanchion-type), access to pastures (present or lack); and clinical signs – present or occurring for last 5 years – that might indicate CLA (superficial abscesses, animals in poor condition, respiratory signs, reproductive failures, mastitis).

### Statistical and epidemiological data analysis

Herds were divided into seropositive and seronegative according to serological test results.

Positive and negative herds were compared with respect to their size and age (time from the establishment of a herd till the moment of the study, given in years). For this purpose the average number of animals and average age of a herd were calculated for both positive and negative herds and compared using t-Student test. Normal distribution of evaluated variables was verified using Kolmogorov-Smirnov test and for uniformity of variances Leven's test was used. Difference of averages was considered significant at significance level 0.05 ( $\alpha = 0.05$ ). Relationship between occurrence of the infection and sex of animals was evaluated with independence test  $\chi^2$ . The results were interpreted in the same manner as in the case of t-Student test. Calculations were carried out using software SPSS®17.0.

Between-herd seroprevalence, within-herd seroprevalence and seroprevalence in sex and age groups were calculated in an ordinary way (Thrusfield 2007). To compare appropriate prevalences u test was used ( $\alpha = 0.05$ ) (Olech and Wieczorek 2002).

Analysis of risk factors was conducted for combined data obtained in both surveys in order to increase sample size and therefore the statistical power of the calculation. For each of the risk factors the prevalence ratio (PR) and its 95% confidence interval was calculated and evaluated (Thrusfield 2007). Appropriate calculations were performed using WinEpi-scope® 2.0. When PR turned out statistically significant, intensity of relationship was evaluated by determination of attributable risk for exposed animals ( $AR_{exp}$ ) (Thrusfield 2007).

## Results

**Seroprevalence** In survey carried out in 1996 antibodies against *C. pseudotuberculosis* were found in 10 herds. No seropositive males were found in any herd where all females were seronegative (Table 1). The between-herd seroprevalence was 13.2% (Fig. 1). The seroprevalence in females was 7.9% and was not statistically significantly different from that of males (prevalence – 7.1%) (Table 2). Within-herd seroprevalence varied from 1.7% to 92.3% (Fig. 2).

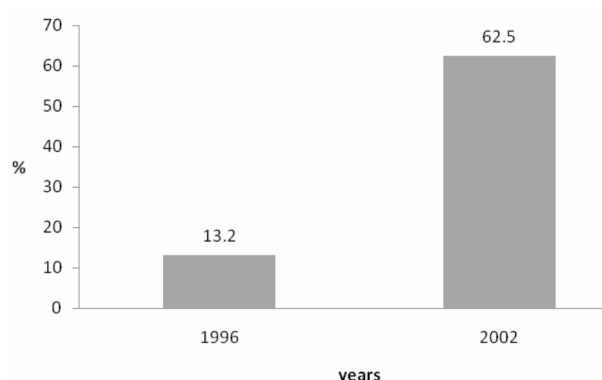


Fig. 1. Between-herd seroprevalence of CLA for herds covered by the milk recording program in Poland in 1996 and 2002.

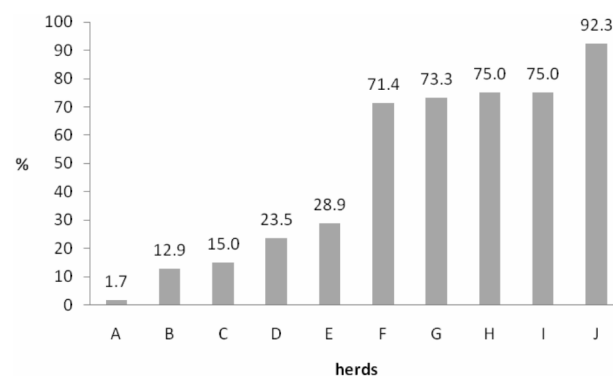


Fig. 2. Within-herd seroprevalence of CLA for seropositive herds in 1996.

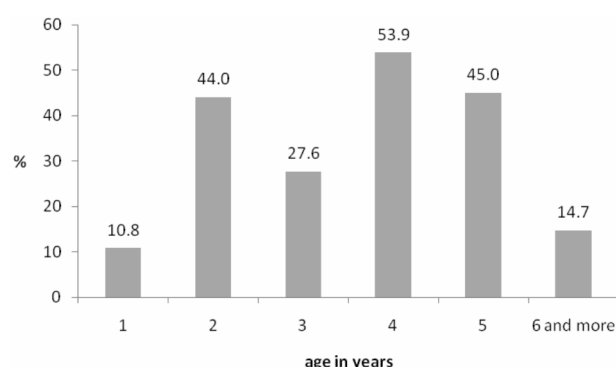


Fig. 3. Distribution of CLA seroprevalence in goats in 1996 according to age of animals

Table 1. Results of serological CLA examination of females and males in particular herds.

| Year of the survey | Serological status of females in a herd | Number of herds where there were: |                         |                         | Total |
|--------------------|---|-----------------------------------|-------------------------|-------------------------|-------|
|                    |   | no males                          | seronegative males only | seropositive males only |       |
| 1996               | All seronegative                        | 27                                | 39                      | 0                       | 66    |
|                    | Seropositive present                    | 1                                 | 5                       | 4                       | 10    |
|                    | Total                                   | 28                                | 44                      | 4                       | 76    |
| 2002               | All seronegative                        | 6                                 | 15                      | 3                       | 24    |
|                    | Seropositive present                    | 11                                | 11                      | 18                      | 40    |
|                    | Total                                   | 17                                | 26                      | 21                      | 64    |

Table 2. Prevalence of CLA for females and males in screened herds.

| Year of the survey | Females                |                            |                   | Males                  |                            |                |
|--------------------|------------------------|----------------------------|-------------------|------------------------|----------------------------|----------------|
|                    | number of goats tested | number of positive results | prevalence (%)    | number of goats tested | number of positive results | prevalence (%) |
| 1996               | 1004                   | 79                         | 7.9               | 70                     | 5                          | 7.1            |
| 2002               | 1386                   | 478                        | 34.5 <sup>a</sup> | 91                     | 36                         | 39.6           |

<sup>a</sup> unreliable result

Table 3. Average number of animals in CLA seronegative and seropositive herds and average age of seronegative and seropositive herds.

| Year of the survey | Average number of animals in a herd |       | Average age of a herd (in years) |      |
|--------------------|-------------------------------------|-------|----------------------------------|------|
|                    | 1996                                | 2002  | 1996                             | 2002 |
| Positive herds     | 91.1*                               | 75.7* | 3.8                              | 7.5  |
| Negative herds     | 14.3*                               | 29.5* | 5.5                              | 8.2  |

\* – statistically significant difference ( $p \leq 0.05$ )

Table 4. Risk factors for CLA in goat herds.

| Risk factors                    | Seropositive herds (%) | Seronegative herds (%) | Prevalence ratio | Lower confidence limit (Logarithmic) | Upper confidence limit (Logarithmic) | Attributable risk <sub>exposed</sub> |
|---------------------------------|------------------------|------------------------|------------------|--------------------------------------|--------------------------------------|--------------------------------------|
| Presence of seropositive males  | 44.0                   | 3.6                    | 3.850*           | 2.445                                | 6.063                                | 0.651                                |
| Superficial abscesses           | 74.0                   | 15.5                   | 6.142*           | 3.357                                | 11.240                               | 0.620                                |
| Respiratory signs               | 54.0                   | 21.4                   | 2.900*           | 1.802                                | 4.666                                | 0.393                                |
| Animals in poor condition       | 34.0                   | 20.2                   | 2.774*           | 1.755                                | 4.386                                | 0.390                                |
| Reproductive failures           | 28.0                   | 15.5                   | 1.798*           | 1.121                                | 2.883                                | 0.230                                |
| Free-stall housing system       | 92.0                   | 86.9                   | 3.479            | 0.924                                | 13.100                               | 0.275                                |
| Purchase of animals from abroad | 16.0                   | 10.7                   | 1.412            | 0.803                                | 2.482                                | 0.137                                |
| Pasture grazing                 | 90.0                   | 92.9                   | 1.585            | 0.572                                | 4.394                                | 0.135                                |
| Mastitis                        | 60.0                   | 58.3                   | 1.410            | 0.831                                | 2.394                                | 0.111                                |
| Poor hygiene conditions         | 40.0                   | 38.1                   | 1.168            | 0.738                                | 1.847                                | 0.055                                |

\* – statistically significant ( $p \leq 0.05$ )

Seroprevalence was the lowest in one-year-old animals. It was higher in two-year-olds, to reach the highest values in four- and five-year-old goats. Seroprevalence in the category of the oldest animals ( $\geq 6$  year-old) was statistically significantly lower than that in any other age class except for the youngest animals (Fig. 3).

In survey carried out in 2002 caseous lymphadenitis was diagnosed in females in 40 herds. In three herds only males were infected, whereas all females were seronegative (Table 1). The between-herd seroprevalence was 62.5% (Fig. 1). The seroprevalence for males was 39.6% (Table 2) and was not statistically significantly different than that of females (approximation of seroprevalence – 34.5%) (Table 2).

### Risk factors

Seropositive herds were significantly larger than seronegative ones (Table 3). No statistically significant difference was shown between the age of seronegative and seropositive herds (Table 3). Analysis of the combined data for years 1996 and 2002 showed that five hypothesized risk factors – presence of seropositive males, abscesses, respiratory signs, animals in poor condition and reproductive failures in a herd – occurred statistically significantly more often in seropositive than in seronegative herds. The remaining four hypothesized risk factors were not statistically significant (Table 4).

### Discussion

Several serological tests for detecting CLA are available (Brown et al. 1986, Brown et al. 1987, Menzies and Muckle 1989, Literak et al. 1995, Dercksen et al. 2000). ELISA is favored due to its high sensitivity and specificity as well as convenience (simplicity, possibility of automation and applicability to large number of samples) (Sutherland et al. 1987, Ter Laak and Shreuder 1991, Menzies et al. 1994, Sting et al. 1998, Binns et al. 2007). In case of CLA, ELISA is of much higher validity in goats than in sheep (Dercksen et al. 2000). The ELISA used in the study had quite high sensitivity and specificity (Kaba et al. 2001) when compared with four other ELISA evaluated by Dercksen et al. (2000).

In our study, large differences in within-herd seroprevalence were observed between herds. As the disease was not a subject to planned control in tested herds, high seroprevalences were likely to result from undisturbed spread of the disease. Very high prevalences exceeding 90%, were also found in the Czech Republic and Brazil (Skalka et al. 1998, Seyffert et al. 2010). Analysis of seroprevalence in particular age

groups shows that the percentage of infected animals grows rapidly with age. While in the youngest animals (up to 1 year) antibodies are hardly ever found (approx. 10%), in one or two-year-old goats seroprevalence exceeds 40%. These data demonstrate the potential of CLA to spread rapidly in a herd, which seems to be confirmed by earlier observations regarding the pathogenesis of the disease (Kuria et al. 2001, Williamson 2001). In animals older than five years, the proportion of seropositive animals significantly decreased. This is likely to be the result of the selection of goats: since infected animals are eliminated from herds earlier, the disease might limit the productive life span of goats to 4-5 years. Nevertheless since no data on duration of antibodies after infection are available, it is possible that antibodies just vanished and hence were absent in animals older than 6 years.

The sampling method applied in 1996 enabled reliable evaluation of seroprevalence for both males and females. In contrast, the survey carried out in 2002 provided reliable data on seroprevalence in males, but only approximate data on females. In 1996 the seroprevalence in both sexes (7.9% in females and 7.1% in males) was not statistically significantly different. In 2002 the seroprevalence in males was 39.6%. It is worth noting that the seroprevalence in females (34.5%), although calculated on the basis of too small sample, remains similar to the value in males (Table 2), which may indicate similarity in the spread of the disease in males and females. As a rule females are commingled with males only during the mating season (for 30-40 days). Very similar seroprevalences in both sex groups indicate that this limited period of contact is sufficient for the spread of the disease. It confirms earlier observations on fast and easy spread of the disease within a herd (Kuria et al. 2001, Williamson 2001).

The present data also allowed evaluation of the disease spread among goat herds. In 1996 specific antibodies were found in 13.2% of herds, but six years later this proportion increased almost five-fold (to 62.5%). Therefore, CLA can spread easily not only within a herd, but also between herds. In the study carried out in Brazil infection was reported in 98% of herds (Seyffert et al. 2010). It may emerge that in Poland an increasing tendency occurs, too. All herds participating in both studies in Poland were distributed throughout the entire country and there was neither direct nor indirect contact between animals. Apparently, the only possible way of introducing the disease into the herd was the purchase of an infected goat (Kuria et al. 2001). Results of our studies indicate that the purchase of animals from abroad is not a statistically significant risk factor. Hence animal trade within the country constitutes the major threat. Breeders are usually aware of hazards resulting from the purchase of new animals and avoid such situations

as far as it is possible. However, they have to replace males in their herds.

Husbandry and breeding practices in Poland involve the same males being used in subsequent seasons in many different herds. This situation is especially common in herds covered by the milk recording program, where only breeding goats can be introduced. The number of herds captured by milk recording as well as the number of breeding males in Poland has never been high. Exchange of male goats between herds is therefore very intensive and it is impossible to eschew this practice, or even limit it. Thus it is very important to evaluate the risk attributed to this practice. The analysis of data on seroprevalence in both sexes demonstrated that, despite limited contacts between goats in Poland, transmission of the disease from males to females occurs. Introducing a seropositive male into a herd constitutes a statistically significant risk factor. The attributable risk equals 0.651 and is the highest of all factors that were studied (Table 4). It is worth noting that in 1996 no infected males were found in seronegative herds, but in 2002 they were found in three herds (almost 13%) (Table 1). This probably reflects the disease spread. Breeders therefore should definitely avoid introducing infected males into their herds, even for the mating season only.

The most characteristic clinical manifestation of CLA in goats is the formation of abscesses in superficial lymph nodes. Apparently presence of abscesses is also a statistically significant risk factor (Table 4). It is also to be expected that the attributable risk should be very high. This circumstance makes the diagnosis much easier (also for breeders) and may significantly limit spread of the disease. In this study  $AR_{exp}$  reaches only 0.620. However, abscesses may result from many other infections (Gezon et al. 1991, Alhendi et al. 1993). This causes  $AR_{exp}$  to be lower than expected. On the other hand, it is worth noticing that abscesses could be seen in only 74.0% of seropositive herds (Table 4). Consequently, purchase of an animal from a herd in which no superficial abscesses have been observed in females not necessarily counters the disease spread.

CLA leads also to the development of other less specific clinical signs. In infected herds respiratory signs, poor condition and reproductive problems are observed more often and they constitute statistically significant risk factors. Respective values of the attributable risk are not high and do not exceed 40% (Table 4), which obviously results from the fact that the aforementioned clinical signs frequently accompany many other diseases.

We did not detect a statistically significant relationship between the presence of CLA in a herd and the occurrence of mastitis in goats. It seems that although such conditions were noticed in screened herds, they tended to occur sporadically. Also hus-

bandry (housing system, pasture grazing, hygiene) was not associated with the presence of the disease.

The disease occurred statistically significantly more often in large herds than in small ones. Large herds are usually formed in Poland by purchasing and commingling animals from many sources and such circumstances facilitate spread of the disease. There was no statistically significant difference in the age of positive and negative herds. The disease occurs therefore in newly established herds as well as in those that have existed for many years. This fact seems to confirm the earlier conclusion on simple and rapid spread of the disease among herds.

Concluding, the study allowed identification of main risk factors for the spread of CLA in a goat population, which were: presence of seropositive males in a herd, presence of superficial abscesses in animals, presence of respiratory signs in animals, presence of animals in poor condition in a herd and occurrence of reproductive failures in a herd.

## Acknowledgements

This work was partially supported by a grant from the State Committee for Scientific Research, number P06K 007 27.

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